Impact of Viral Infection on Absorption and Scattering Properties of Marine Bacteria and Phytoplankton

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LONG-TERM GOALS

The long-range goal of our research is to document the role of viruses in light scattering in the sea. The current DEPSCoR project is a joint venture between the University of New England and its affiliate, Bigelow Laboratory for Ocean Sciences.

OBJECTIVES

The objectives of this work are to: 1) define the typical time scale for the shift in inherent optical properties (a, b, c, and bb) associated with viral infection of marine bacteria (as compared to non infected control populations), 2) define the maximum possible rates of shift in inherent optical properties (a, b, c, and bb) under conditions of high virus multiplicity of infection, and 3) define the concurrent changes in 0.03-100 μm size spectra associated with viral infection of marine bacteria.
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APPROACH

This work involves a significant number of laboratory experiments leading to mesocosm experiments off of the Bigelow Dock (West Boothbay Harbor, ME). The latter experiments provide adequate sample volume to make all size spectrum, bb, and cell count measurements, plus the ac-9 measurements of a and c (which require larger volumes). Total scattering will be calculated as the difference between c and a. By using large volumes with known host and controlled virus addition, we can be assured that 1) inoculated viruses are host specific, 2) host culturing conditions are optimal for virus assays, 3) host growth conditions are sufficiently understood from preliminary experiments in aqueous media, 4) initial viral concentrations are easily set, 5) bottle effects are minimized because of

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14. ABSTRACT
The long-range goal of our research is to document the role of viruses in light scattering in the sea. The current DEPSCoR project is a joint venture between the University of New England and its affiliate, Bigelow Laboratory for Ocean Sciences.

15. SUBJECT TERMS

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the large volumes, and 6) mesocosms provide a realistic scale for observing viral infection dynamics in blooms of marine organisms. In our review of the literature, we have observed many cases where strict controls were not run such that results were too ambiguous to interpret.

WORK COMPLETED

Our methods for isolation and enumeration of marine viruses and host cells have been described elsewhere (Adams 1959; Noble and Fuhrman 1998; Balch et al. 2000). Size distribution of sub-micron particles was measured with FFFF (FFFractionation, LLC, UT), a chromatographic-like separation technique (Vaillancourt and Balch, 2000). Volume scattering of the bacterial/virus suspensions was measured with a Dawn Laser Light Scattering Photometer (Wyatt Technologies, CA) 2000 times over 10s(Balch et al.1999; Balch et al. 2000) (samples were not mixed during this period). Viruses from purified concentrates were viewed with a Zeiss Transmission Electron Microscope (model 902A).

Infection experiments were performed by adding diluted host bacteria to two sterile, borosilicate, screw-cap vials. Host were always derived from log phase cultures and maintained at 25C. Viruses were added to one of the vials using a multiplicity of infection (MOI) of 1.0, as determined by pre-experimental counts. At regular intervals, samples were withdrawn for volume scattering measurement, bacteria/virus enumeration (using plaque/plate counts and Sybr-Green counts (Noble and Fuhrman 1998)) and FFFF analysis. Vials were then returned to reduced light conditions at 25C until the next sampling.

A mesocosm experiment was conducted using *P. perfectomarina*, grown outdoors under environmentally relevant conditions of temperature and light, using 100 liter, autoclavable, plastic bags, to examine changes in bb and attenuation after infection. The experimental design included control (no viruses added) and experimental (infected) bags. The bags transmitted light as follows: >90% from 300-700nm, 85% at 250nm and 25% at 200nm. Bags, fitted with a sampling tube, were autoclaved prior to use and filled with 100 l of sterile media (f/2+0.05% yeast extract) via a peristaltic pump. To protect bags from wave action, each was tethered inside an oper topped, 208 l polyethylene cylindrical tank which was extensively perforated with 2cm holes to let ambient seawater freely circulate. Media in the bags was allowed to equilibrate for 20h prior to initiation of the experiment. Host was added to both bags to an initial concentration of 10^7 per ml. LS-05 virus was added to the experimental bag 2h later. One-liter aliquots were removed periodically for optical measurements and particle counts. Samples were run (via peristaltic pump) through a Wet Labs (Philomath, OR) ac-9, to measure absorption (a) and attenuation (c) at 9 visible wavelengths (Mueller and Austin, 1995), for the total suspensions and the fraction passing a 0.2μm cartridge filter (colored dissolved organic material).

RESULTS

In all cases, viral infection caused a pronounced change in the light scattering properties of the marine bacterial populations. Qualitatively, the turbidity of control and infected samples remained the same for several hours, after which infected samples were rapidly clarified. Relative to turbid controls, infected cultures were as clear as media blanks. Quantitatively, turbidity loss was observed as a sharp drop in the backscattering coefficients, attributable to two factors: 1) a distinct decrease in volume scattering values, and 2) for three host species (*Vibrio harveyii, Psuedomonas perfectomarina*, and *Photobacterium* sp.), a pronounced flattening of the VSF in the forward and backward directions. Accompanying these changes were simultaneous increases in virus concentrations in infected samples, and decreases in host bacteria after a short infection period. Uninfected bacteria exhibited standard logarithmic growth followed by a stationary phase. Flow field-flow fractionation (FFFF) revealed the

presence of peaks close to, but not necessarily identical to (Vaillancourt and Balch 2000), the virus size as estimated by transmission electron microscopy (TEM).

Infection of cyanobacterium (*Synechococcus* sp. Bigelow CCMP #1333 and 1334) also resulted in reductions in turbidity. After 6d of infection, backscattering decreased to 0.03m^{-1}, significantly clearer than the original media blank. In addition, the infected sample lost its characteristic green (CCMP 1333), or red (CCMP 1334) reflectance (observable in the control), and large aggregates of cellular debris appeared at the bottom of the vessel. Volume scattering of infected samples was markedly lower than the controls, but no significant changes in VSF shape were detectable due to low signal to noise in these particular measurements.

In the outdoor mesocosm experiment with LS-05 virus and its host, *P. perfectomarina*, phage concentration increased ~1000X after 24h, then it stabilized at $10^{10}ml^{-1}$. Host cell concentration in the infected bag increased more slowly than in the control and no sharp decreases in bacterial abundance were noted except 5h after infection. The most notable change was an increase in beam attenuation (c) of the <0.2μm "dissolved" material at 412nm from 30 to 46h. Increased c of the dissolved material was associated with an increase in phage concentration ~24h earlier. Spectral attenuation of this cDOM (after correction for the media blank) decreased exponentially with wavelength (least-squares fit equation: ccDOM λ =ccDOM 412 exp[-0.0043(l-412)]; r2 =0.99; data not shown). FFFF data showed an initial peak at the same particle size as previous infection experiments with LS-05 phage (90nm), but 46h after infection, where a secondary broad peak developed at 450nm.

The most notable result from these experiments was the rapidity with which turbid, marine bacteria suspensions were clarified following viral infection, directly attributed to decreases in volume scattering and flattening of the VSF. Clearing times of 1-5h were common for heterotrophic host species, while the cyanobacterial host required 6d to clear (with the actual clearing process confined to ~30h). In the context of optical changes in the sea, clearing of infected bacterial suspensions is the same, or faster than increases in turbidity due to cell growth. Our results are most applicable to phage infection of a low diversity microbial population; the magnitude of clearing likely would be inversely proportional to the diversity of the assemblage.

It is useful to evaluate the contribution of viruses per se to the observed backscattering. Virus concentrations in the sea range from 10^3 to 10^{10} l^{-1} in eutrophic regions (Bergh et al. 1989; Bratbak et al. 1993; Bratbak et al. 1996; Fuhrman 1999). Our experiments began with bacteriophage concentrations which were typical for eutrophic areas (10^9-$10^{10}l^{-1}$). Typically, viral concentrations approached ~ 10^{13} $liter^{-1}$ at the conclusion of each experiment. Observed backscattering cross-sections of viruses were comparable to those measured previously (Balch et al. 2000). Thus, in most cases, concentrations of viruses used in our experiments increased backscattering by a negligible amount (~0.01 m^{-1}). For example, K-5 and LS-05 viruses contributed ~10% of the final backscattering; the remainder likely came from host fragments, or aggregates. Thus, extrapolated to field conditions, phage contribution to backscattering is expected to be minimal at 10^{10} l^{-1} and the largest optical effect is associated with lysis of optically active hosts and subsequent release of intracellular organic debris. Based on attenuation measurements from the mesocosm experiment, it appears that the resulting optical shift during infection was associated with a release of blue-attenuating, dissolved material from the host (which passed through a 0.2μm filter). This suggests a shift from particle scattering, dominated by intact cells, to molecular scattering, also known as "Einstein-Smoluchowski" or "Rayleigh" scattering (Mobley 1992), which is dominated by particles smaller than the wavelength of light. Interestingly, the increase in dissolved attenuation (implying release of cDOM from host cells), lagged the increase in viral concentration by ~24h, suggesting that the optical change of the

suspension, while likely caused by the host lysis, involved some process in addition to simple leakage of cDOM. Further clues to this alternate process were seen in the FFFF results, that showed a 0.09µm peak in the infected sample 26h after infection. At 46h, the "dissolved" fraction showed an additional particle peak at 0.45µm). We suspect such particles were sufficiently flexible to pass through the 0.2µm pore filter, and cause the increase in blue attenuation. The time scale for appearance of these 0.45µm particles suggests they might have been polymer gels (Chin et al.1998; Orellana and Verdugo 1999), assembled from smaller polymers released during host lysis 24h earlier. This is consistent with previous observations in which assembly of 0.5µm diameter polymer gels occurred after ~12h (Chin et al.1998).

IMPACT/APPLICATIONS

These are the first quantitative optical observations of phage-induced clarification of marine bacterial suspensions. While viruses themselves contribute little to backscattering, their infection and lysis of bacterioplankton can rapidly alter light scattering. Our observations extend the understanding of the important role of viruses in the turnover of plankton populations (Fuhrman 1999) and the optical consequences of their infection. Monospecific blooms of prokaryotic or eukaryotic algae are well known (Balch et al. 1991; Bidigare et al. 1997; Morel 1997) and their viral control has been implicated (Bratbak et al. 1993; Bratbak et al. 1995; Bratbak et al. 1996; Brussaard et al. 1996). We hypothesize that viral infection of optically-active blooms provides an ideal mechanism for: 1) rapid reduction in turbidity due to profound changes in the VSFs of the particle suspension (as the scattering of the suspension changes from particle-scattering-dominated to molecular scattering-dominated), 2) changes in particle and dissolved absorption as cells release cDOM, and 3) aggregation of DOM into "Koike" particles"/ polymer-gels (Koike et al. 1990; Chin et al. 1998; Wells 1998), themselves a potentially important source of scattering (Stramski and Kiefer 1991).

TRANSITIONS

We have begun propagating several additional species of Synechococcus and other photosynthetic bacteria, obtained from the Bigelow Provasoli-Guillard Center for the Culture of Marine Phytoplankton. These will be be used during the fall to recover species-specific virus strains from Maine coastal waters. Once isolated, virus and host cultures will be used in optical experiments similar to those described above. Accompanying these will be a series of descriptive studies of new virus isolates including purification, concentration, Electron Microscopy, and nucleic acid typing. It is expected that purified, isolate concentrates will be subjected to the type of optical analyses described in our first ONR project (Optical Properties of Viruses). Additional outdoor mesocosm experiments are planned for the spring.

RELATED PROJECTS

This DEPSCoR work is a joint venture between the University of New England and its affiliate Bigelow Laboratory. Collaborative relationships are maintained with Dr. Ken Voss and Dr. Howard Gordon, both ONR-funded investigators at the University of Miami Dept. of Physics. This work is the logical outgrowth of a recently completed DEPSCoR project (N000149801999).

Figure 1-Control (left) and experimental (right) flasks of Synechococcus strain 1333, taken 12d after experimental flask was infected by purified Synechococcus virus. Flasks were illuminated from below to highlight the intense green reflectance of the uninfected control flask, and the greater transparency and loss of turbidity in the infected experimental flask. Note also the presence of aggregate particles in the experimental flask.

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